



71914.ST25.txt
SEQUENCE LISTING

<110> COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH

<120> STABLE GENE VARIANTS OF LIPASES

<130> 71914

<140> US 10/768,951

<141> 2004-01-29

<160> 22

<170> PatentIn version 3.3

<210> 1

<211> 181

<212> PRT

<213> Bacillus subtilis

<220>

<221> AMINO ACIDS

<222> (1)..(181)

<223> enzyme sequence

<400> 1

Met Ala Glu His Asn Pro Val Val Met Val His Gly Ile Gly Gly Ala
1 5 10 15

Ser Phe Asn Phe Ala Gly Ile Lys Ser Tyr Leu Val Ser Gln Gly Trp
20 25 30

Ser Arg Asp Lys Leu Tyr Ala Val Asp Phe Trp Asp Lys Thr Gly Thr
35 40 45

Asn Tyr Asn Asn Gly Pro Val Leu Ser Arg Phe Val Gln Lys Val Leu
50 55 60

Asp Glu Thr Gly Ala Lys Lys Val Asp Ile Val Ala His Ser Met Gly
65 70 75 80

Gly Ala Asn Thr Leu Tyr Tyr Ile Lys Asn Leu Asp Gly Gly Asn Lys
85 90 95

Val Ala Asn Val Val Thr Leu Gly Gly Ala Asn Arg Leu Thr Thr Gly
100 105 110

Lys Ala Leu Pro Gly Thr Asp Pro Asn Gln Lys Ile Leu Tyr Thr Ser
115 120 125

Ile Tyr Ser Ser Ala Asp Met Ile Val Met Asn Tyr Leu Ser Arg Leu
130 135 140

71914.ST25.txt

Asp Gly Ala Arg Asn Val Gln Ile His Gly Gly His Ile Gly Leu Leu
145 150 155 160

Tyr Ser Ser Gln Val Asn Ser Leu Ile Lys Glu Gly Leu Asn Gly Gly
165 170 175

Gly Gln Asn Thr Asn
180

<210> 2
<211> 181
<212> PRT
<213> Bacillus subtilis

<220>
<221> Amino acid
<222> (1)..(181)
<223> Protein sequence

<400> 2

Met Ala Glu His Asn Pro Val Val Met Val His Gly Ile Gly Gly Ala
1 5 10 15

Ser Phe Asn Phe Ala Gly Ile Lys Ser Tyr Leu Val Ser Gln Gly Trp
20 25 30

Ser Arg Asp Lys Leu Tyr Ala Val Asp Phe Trp Asp Lys Thr Gly Thr
35 40 45

Asn Tyr Asn Asn Gly Pro Val Leu Ser Arg Phe Val Gln Lys Val Leu
50 55 60

Asp Glu Thr Gly Ala Lys Lys Val Asp Ile Val Ala His Ser Met Gly
65 70 75 80

Gly Ala Asn Thr Leu Tyr Tyr Ile Lys Asn Leu Asp Gly Gly Asn Lys
85 90 95

Val Ala Asn Val Val Thr Leu Gly Gly Ala Asn Arg Leu Thr Thr Gly
100 105 110

Lys Ala Leu Pro Gly Thr Asp Pro Asn Gln Lys Ile Leu Tyr Thr Ser
115 120 125

Ile Tyr Ser Ser Ala Asp Met Ile Val Met Asn Tyr Leu Ser Arg Leu
130 135 140

Asp Gly Ala Arg Asn Val Gln Ile His Gly Gly His Ile Gly Leu Leu
Page 2

145 150 160

Tyr Ser Ser Gln Val Tyr Ser Leu Ile Lys Glu Gly Leu Asn Gly Gly
165 170 175

Gly Gln Asn Thr Asn
180

<210> 3
<211> 181
<212> PRT
<213> Bacillus subtilis

<220>
<221> Amino acid
<222> (1)..(181)
<223> Protein sequence

<400> 3

Met Ala Glu His Asn Pro Val Val Met Val His Gly Ile Gly Gly Ala
1 5 10 15

Ser Phe Asn Phe Ala Gly Ile Lys Ser Tyr Leu Val Ser Gln Gly Trp
20 25 30

Ser Arg Asp Lys Leu Tyr Ala Val Asp Phe Trp Asp Lys Thr Gly Thr
35 40 45

Asn Tyr Asn Asn Gly Pro Val Leu Ser Arg Phe Val Gln Lys Val Leu
50 55 60

Asp Glu Thr Gly Val Lys Lys Val Asp Ile Val Ala His Ser Met Gly
65 70 75 80

Gly Ala Asn Thr Leu Tyr Tyr Ile Lys Asn Leu Asp Gly Gly Asn Lys
85 90 95

Val Ala Asn Val Val Thr Leu Gly Gly Ala Asn Arg Leu Thr Thr Gly
100 105 110

Lys Ala Leu Pro Gly Thr Asp Pro Asn Gln Lys Ile Leu Tyr Thr Ser
115 120 125

Ile Tyr Ser Ser Asp Asp Met Ile Val Met Asn Tyr Leu Ser Arg Leu
130 135 140

Asp Gly Ala Arg Asn Val Gln Ile His Gly Gly His Ile Gly Leu Leu
145 150 155 160

71914.ST25.txt

Tyr Ser Ser Gln Val Tyr Ser Leu Ile Lys Glu Gly Leu Asn Gly Gly
165 170 175

Gly Gln Asn Thr Asn
180

<210> 4
<211> 181
<212> PRT
<213> Bacillus subtilis

<220>
<221> Amino acid
<222> (1)..(181)
<223> Protein Sequence

<400> 4

Met Ala Glu His Asn Pro Val Val Met Val His Gly Ile Gly Gly Ala
1 5 10 15

Ser Phe Asn Phe Ala Gly Ile Lys Ser Tyr Leu Val Ser Gln Gly Trp
20 25 30

Ser Arg Asp Lys Leu Tyr Ala Val Asp Phe Trp Asp Lys Thr Gly Thr
35 40 45

Asn Tyr Asn Asn Gly Pro Val Leu Ser Arg Phe Val Gln Lys Val Leu
50 55 60

Asp Glu Thr Gly Thr Lys Lys Val Asp Ile Val Ala His Ser Met Gly
65 70 75 80

Gly Ala Asn Thr Leu Tyr Tyr Ile Lys Asn Leu Asp Gly Gly Asn Lys
85 90 95

Val Ala Asn Val Val Thr Leu Gly Gly Ala Asn Arg Leu Thr Thr Gly
100 105 110

Lys Ala Pro Pro Gly Thr Asp Pro Asn Gln Lys Ile Leu Tyr Thr Ser
115 120 125

Ile Tyr Ser Ser Ala Asp Met Ile Val Met Asn Tyr Leu Ser Arg Leu
130 135 140

Asp Gly Ala Arg Asn Val Gln Ile His Gly Gly His Ile Gly Leu Leu
145 150 155 160

Tyr Ser Ser Gln Val Tyr Ser Leu Ile Lys Glu Gly Leu Asn Gly Gly
Page 4

Gly Gln Asn Thr Asn
180

<210> 5
<211> 181
<212> PRT
<213> Bacillus subtilis

<220>
<221> Amino acid
<222> (1)..(181)
<223> Protein Sequence

<400> 5

Met Ala Glu His Asn Pro Val Val Met Val His Gly Ile Gly Gly Ala
1 5 10 15

Ser Phe Asn Phe Ala Gly Ile Lys Ser Tyr Leu Val Ser Gln Gly Trp
20 25 30

Ser Arg Asp Lys Leu Tyr Ala Val Asp Phe Trp Asp Lys Thr Gly Thr
35 40 45

Asn Tyr Asn Asn Gly Pro Val Leu Ser Arg Phe Val Gln Lys Val Leu
50 55 60

Asp Glu Thr Gly Ala Lys Lys Val Asp Ile Val Ala His Ser Met Gly
65 70 75 80

Gly Ala Asn Thr Leu Tyr Tyr Ile Lys Asn Leu Asp Gly Gly Asn Lys
85 90 95

Val Ala Asn Val Val Thr Leu Gly Gly Ala Asn Arg Leu Thr Thr Gly
100 105 110

Lys Ala Leu Pro Gly Thr Asp Pro Asn Gln Lys Ile Leu Tyr Thr Ser
115 120 125

Ile Tyr Ser Ser Asp Asp Met Ile Val Met Asn Tyr Leu Ser Arg Leu
130 135 140

Asp Gly Ala Arg Asn Val Gln Ile His Gly Gly His Ile Gly Leu Leu
145 150 155 160

Tyr Ser Ser Gln Val Tyr Ser Leu Ile Lys Glu Gly Leu Asn Gly Gly
165 170 175

Gly Gln Asn Thr Asn
180

<210> 6
<211> 181
<212> PRT
<213> Bacillus subtilis

<220>
<221> Amino acid
<222> (1)..(181)
<223> Protein sequence

<400> 6

Met Ala Glu His Asn Pro Val Val Met Val His Gly Ile Gly Gly Ala
1 5 10 15

Ser Phe Asn Phe Ala Gly Ile Lys Ser Tyr Leu Val Ser Gln Gly Trp
20 25 30

Ser Arg Asp Lys Leu Tyr Ala Val Asp Phe Trp Asp Lys Thr Gly Thr
35 40 45

Asn Tyr Asn Asn Gly Pro Val Leu Ser Arg Phe Val Gln Lys Val Leu
50 55 60

Asp Glu Thr Gly Ala Lys Lys Val Asp Ile Val Ala His Ser Met Gly
65 70 75 80

Gly Ala Asn Thr Leu Tyr Tyr Ile Lys Asn Leu Asp Gly Gly Asn Lys
85 90 95

Val Ala Asn Val Val Thr Leu Gly Gly Ala Asn Arg Leu Thr Thr Gly
100 105 110

Lys Ala Pro Pro Gly Thr Asp Pro Asn Gln Lys Ile Leu Tyr Thr Ser
115 120 125

Ile Tyr Ser Ser Asp Asp Met Ile Val Met Asn Tyr Leu Ser Arg Leu
130 135 140

Asp Gly Ala Arg Asn Val Gln Ile His Gly Gly His Ile Gly Leu Leu
145 150 155 160

Tyr Ser Ser Gln Val Tyr Ser Leu Ile Lys Glu Gly Leu Asn Gly Gly
165 170 175

Gly Gln Asn Thr Asn

180

<210> 7
 <211> 181
 <212> PRT
 <213> Bacillus subtilis

<220>
 <221> Amino acid
 <222> (1)..(181)
 <223> Protein sequence

<400> 7

Met Ala Glu His Asn Pro Val Val Met Val His Gly Ile Gly Gly Ala
 1 5 10 15

Ser Phe Asn Phe Ala Gly Ile Lys Ser Tyr Leu Val Ser Gln Gly Trp
 20 25 30

Ser Arg Asp Lys Leu Tyr Ala Val Asp Phe Trp Asp Lys Thr Gly Thr
 35 40 45

Asn Tyr Asn Asn Gly Pro Val Leu Ser Arg Phe Val Gln Lys Val Leu
 50 55 60

Asp Glu Thr Gly Ala Lys Lys Val Asp Ile Val Ala His Ser Met Gly
 65 70 75 80

Gly Ala Asn Thr Leu Tyr Tyr Ile Lys Asn Leu Asp Gly Gly Asn Lys
 85 90 95

Val Ala Asn Val Val Thr Leu Gly Gly Ala Asn Arg Leu Thr Thr Gly
 100 105 110

Lys Ala Leu Pro Gly Thr Asp Pro Asn Gln Lys Ile Leu Tyr Thr Ser
 115 120 125

Ile Tyr Ser Ser Ala Asp Met Ile Val Met Asn Tyr Leu Ser Arg Leu
 130 135 140

Asp Gly Ala Ser Asn Val Gln Ile His Gly Gly His Ile Gly Leu Leu
 145 150 155 160

Tyr Ser Ser Gln Val Tyr Ser Leu Ile Lys Glu Gly Leu Asn Gly Gly
 165 170 175

Gly Gln Asn Thr Asn
 180

<210> 8
 <211> 181
 <212> PRT
 <213> Bacillus subtilis

<220>
 <221> Amino acid
 <222> (1)..(181)
 <223> Protein sequence

<400> 8

Met Ala Glu His Asn Pro Val Val Met Val His Gly Ile Gly Gly Ala
 1 5 10 15

Ser Phe Asn Phe Ala Gly Ile Lys Ser Tyr Leu Val Ser Gln Gly Trp
 20 25 30

Ser Arg Asp Lys Leu Tyr Ala Val Asp Phe Trp Asp Lys Thr Gly Thr
 35 40 45

Asn Tyr Asn Asn Gly Pro Val Leu Ser Arg Phe Val Gln Lys Val Leu
 50 55 60

Asp Glu Thr Gly Ala Lys Lys Val Asp Ile Val Ala His Ser Met Gly
 65 70 75 80

Gly Ala Asn Thr Leu Tyr Tyr Ile Lys Asn Leu Asp Gly Gly Asn Lys
 85 90 95

Val Ala Asn Val Val Thr Leu Gly Gly Ala Asn Arg Leu Thr Thr Gly
 100 105 110

Lys Ala Pro Pro Gly Thr Asp Pro Asn Gln Lys Ile Leu Tyr Thr Ser
 115 120 125

Ile Tyr Ser Ser Ala Asp Met Ile Val Met Asn Tyr Leu Ser Arg Leu
 130 135 140

Asp Gly Ala Arg Asn Val Gln Ile His Gly Gly His Ile Gly Leu Leu
 145 150 155 160

Tyr Ser Ser Gln Val Tyr Ser Leu Ile Lys Glu Gly Leu Asn Gly Gly
 165 170 175

Gly Gln Asn Thr Asn
 180

<210> 9

<211> 181
 <212> PRT
 <213> Bacillus subtilis

<220>
 <221> Amino acid
 <222> (1)..(181)
 <223> Protein Sequence

<400> 9

Met Ala Glu His Asn Pro Val Val Met Val His Gly Ile Gly Gly Ala
 1 5 10 15

Ser Phe Asn Phe Ala Gly Ile Lys Ser Tyr Leu Val Ser Gln Gly Trp
 20 25 30

Ser Arg Asp Lys Leu Tyr Ala Val Asp Phe Trp Asp Lys Thr Gly Thr
 35 40 45

Asn Tyr Asn Asn Gly Pro Val Leu Ser Arg Phe Val Gln Lys Val Leu
 50 55 60

Asp Glu Thr Gly Ala Lys Lys Ala Asp Ile Val Ala His Ser Met Gly
 65 70 75 80

Gly Ala Asn Thr Leu Tyr Tyr Ile Lys Asn Leu Asp Gly Gly Asn Lys
 85 90 95

Val Ala Asn Val Val Thr Leu Gly Gly Ala Asn Arg Leu Thr Thr Gly
 100 105 110

Lys Ala Leu Pro Gly Thr Asp Pro Asn Gln Lys Ile Leu Tyr Thr Ser
 115 120 125

Ile Tyr Ser Ser Ala Asp Met Ile Val Met Asn Tyr Leu Ser Arg Leu
 130 135 140

Asp Gly Ala Arg Asn Val Gln Ile His Gly Gly His Ile Gly Leu Leu
 145 150 155 160

Tyr Ser Ser Gln Val Tyr Ser Leu Ile Lys Glu Gly Leu Asn Gly Gly
 165 170 175

Gly Gln Asn Thr Asn
 180

<210> 10
 <211> 181
 <212> PRT

<213> Bacillus subtilis

<220>

<221> Amino acid

<222> (1)..(181)

<223> Protein Sequence

<400> 10

Met Ala Glu His Asn Pro Val Val Met Val His Gly Ile Gly Gly Ala
1 5 10 15

Ser Phe Asn Phe Ala Gly Ile Lys Ser Tyr Leu Val Ser Gln Gly Trp
20 25 30

Ser Arg Asp Lys Leu Tyr Ala Val Asp Phe Trp Asp Lys Thr Gly Thr
35 40 45

Asn Tyr Asn Asn Gly Pro Val Leu Ser Arg Phe Val Gln Lys Val Leu
50 55 60

Asp Glu Thr Gly Ala Lys Lys Val Asp Ile Val Ala His Ser Met Gly
65 70 75 80

Gly Ala Asn Thr Leu Tyr Tyr Ile Lys Asn Leu Asp Gly Gly Asn Lys
85 90 95

Val Ala Asn Val Val Thr Leu Gly Gly Ala Asn Arg Leu Thr Thr Gly
100 105 110

Lys Ala Leu Pro Gly Thr Asp Pro Asn Gln Lys Ile Leu Tyr Thr Ser
115 120 125

Ile Tyr Ser Ser Ala Asp Met Ile Val Met Asn Tyr Leu Ser Arg Leu
130 135 140

Val Gly Ala Arg Asn Val Gln Ile His Gly Gly His Ile Gly Leu Leu
145 150 155 160

Tyr Ser Ser Gln Val Tyr Ser Leu Ile Lys Glu Gly Leu Asn Gly Gly
165 170 175

Gly Gln Asn Thr Asn
180

<210> 11

<211> 181

<212> PRT

<213> Bacillus subtilis

<220>
 <221> Amino acid
 <222> (1)..(181)
 <223> Protein sequence

<400> 11

Met Ala Glu His Asn Pro Val Val Met Val His Gly Ile Gly Gly Ala
 1 5 10 15

Ser Phe Asn Phe Ala Gly Ile Lys Ser Tyr Leu Val Ser Gln Gly Trp
 20 25 30

Ser Arg Asp Lys Leu Tyr Ala Val Asp Phe Trp Asp Lys Thr Gly Thr
 35 40 45

Asn Tyr Asn Asn Gly Pro Val Leu Ser Arg Phe Val Gln Lys Val Leu
 50 55 60

Asp Glu Thr Gly Val Lys Lys Val Asp Ile Val Ala His Ser Met Gly
 65 70 75 80

Gly Ala Asn Thr Leu Tyr Tyr Ile Lys Asn Leu Asp Gly Gly Asn Lys
 85 90 95

Val Ala Asn Val Val Thr Leu Gly Gly Ala Asn Arg Leu Thr Thr Gly
 100 105 110

Lys Ala Leu Pro Gly Thr Asp Pro Asp Gln Lys Ile Leu Tyr Thr Ser
 115 120 125

Ile Tyr Ser Ser Asp Asp Met Ile Val Met Asn Tyr Leu Ser Arg Leu
 130 135 140

Asp Gly Ala Arg Asn Val Gln Ile His Gly Gly His Ile Gly Leu Leu
 145 150 155 160

Tyr Ser Ser Gln Val Tyr Ser Leu Ile Lys Glu Gly Leu Asn Gly Gly
 165 170 175

Gly Gln Asn Thr Asn
 180

<210> 12
 <211> 181
 <212> PRT
 <213> Bacillus subtilis

<220>

<221> Amino acid
 <222> (1)..(181)
 <223> Protein sequence

<400> 12

Met Ala Glu His Asn Pro Val Val Met Val His Gly Ile Gly Gly Ala
 1 5 10 15

Ser Phe Asn Phe Ala Gly Ile Lys Ser Tyr Leu Val Ser Gln Gly Trp
 20 25 30

Ser Arg Asp Lys Leu Tyr Ala Val Asp Phe Trp Asp Lys Thr Gly Thr
 35 40 45

Asn Tyr Asn Asn Gly Pro Val Leu Ser Arg Phe Val Gln Lys Val Leu
 50 55 60

Asp Glu Thr Gly Ala Lys Lys Val Asp Ile Val Ala His Ser Met Gly
 65 70 75 80

Gly Ala Asn Thr Leu Tyr Tyr Ile Lys Asn Leu Asp Gly Gly Asn Lys
 85 90 95

Val Ala Asn Val Val Thr Leu Gly Gly Ala Asn Arg Leu Thr Thr Gly
 100 105 110

Lys Ala Pro Pro Gly Thr Asp Pro Asp Gln Lys Ile Leu Tyr Thr Ser
 115 120 125

Ile Tyr Ser Ser Asp Asp Met Ile Val Met Asn Tyr Leu Ser Arg Leu
 130 135 140

Asp Gly Ala Arg Asn Val Gln Ile His Gly Gly His Ile Gly Leu Leu
 145 150 155 160

Tyr Ser Ser Gln Val Tyr Ser Leu Ile Lys Glu Gly Leu Asn Gly Gly
 165 170 175

Gly Gln Asn Thr Asn
 180

<210> 13
 <211> 24
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> oligonucleotide

<400> 13

cgccagggtt ttcccagtca cgac	71914.ST25.txt	24	
<210> 14 <211> 22 <212> DNA <213> Artificial Sequence <220> <223> oligonucleotide <400> 14 tgacacagga aacagctatg ac			22
<210> 15 <211> 28 <212> DNA <213> Artificial Sequence <220> <223> Primer <400> 15 ccatgattac gcatatggct gaacacaa			28
<210> 16 <211> 26 <212> DNA <213> Artificial Sequence <220> <223> Primer <400> 16 ggaggatcat atgaaatttg taaaaa			26
<210> 17 <211> 23 <212> DNA <213> Artificial Sequence <220> <223> Primer <400> 17 cccgggatcc attgtccggt acc			23
<210> 18 <211> 25 <212> DNA <213> Artificial Sequence <220> <223> Primer <400> 18 cgtcagcgaa ttccgctgaa cacat			25

<210> 19
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 19
gcgggaagga tccgaattcg agct

24

<210> 20
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 20
cgccagggtt ttcccagtca cgac

24

<210> 21
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 21
tgacacagga aacagctatg ac

22

<210> 22
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 22
ggcaaggcgc ctccgggaac agat

24